of adding the detergent CTAB to invasive cleavage reactions in which 150 mM LiCl was used in place of the KCl in otherwise standard reactions. Lane 1 shows unreacted (*i.e.*, uncut) probe, and the reaction shown in lane 1 is the LiCl-modified standard reaction without CTAB. The reactions analyzed in lanes 3 and 4 contained 100 μM CTAB, lanes 5 and 6 contained 200 μM CTAB, lanes 7 and 8 contained 400 μM CTAB, lanes 9 and 10 contained 600 μM CTAB, lanes 11 and 12 contained 800 μM CTAB and lanes 13 and 14 contained 1 mM CTAB. These results showed that the lower amounts of CTAB may have a very moderate enhancing effect under these reaction conditions, and the presence of CTAB in excess of about 500 μM was inhibitory to the accumulation of specific cleavage product.

h) Effect Of PEG Addition

Figure 48 shows the effect of adding polyethylene glycol (PEG) at various percentage (w/v) concentrations to otherwise standard reactions. The effects of increasing the reaction temperature of the PEG-containing reactions was also examined. The reactions assayed in lanes 1 and 2 were the standard conditions without PEG, lanes 3 and 4 contained 4% PEG, lanes 5 and 6 contained 8% PEG and lanes 7 and 8 contained 12% PEG. Each of the aforementioned reactions was performed at 61°C. The reactions analyzed in lanes 9, 10, 11 and 12 were performed at 65 °C, and contained 0%, 4%, 8% and 12% PEG, respectively. These results show that at all percentages tested, and at both temperatures tested, the inclusion of PEG substantially eliminated the production of specific cleavage product.

In addition to the data presented above (*i.e.*, effect of CTAB and PEG addition), the presence of 1X Denhardts in the reaction mixture was found to have no adverse effect upon the cleavage reaction [50X Denhardt's contains per 500 ml: 5 g Ficoll, 5 g polyvinylpyrrolidone, 5 g BSA]. In addition , the presence of each component of Denhardt's was examined individually (*i.e.*, Ficoll alone, polyvinylpyrrolidone alone, BSA alone) for the effect upon the invader-directed cleavage reaction; no adverse effect was observed.

i) Effect Of The Addition Of Stabilizing Agents

Another approach to enhancing the output of the invasive cleavage reaction is to enhance the activity of the enzyme employed, either by increasing its stability in the reaction environment or by increasing its turnover rate. Without regard to the precise mechanism by which various agents operate in the invasive cleavage reaction, a number of agents commonly used to stabilize enzymes during prolonged storage were tested for the ability to enhance the accumulation of specific cleavage product in the invasive cleavage reaction.

Figure 49 shows the effects of adding glycerol at 15% and of adding the detergents Tween-20 and Nonidet-P40 at 1.5%, alone or in combination, in otherwise standard reactions. The reaction analyzed in lane 1 was a standard reaction. The reaction analyzed in lane 2 contained 1.5% NP-40, lane 3 contained 1.5% Tween 20, lane 4 contained 15% glycerol. The reaction analyzed in lane 5 contained both Tween-20 and NP-40 added at the above concentrations, lane 6 contained both glycerol and NP-40, lane 7 contained both glycerol and Tween-20, and lane 8 contained all three agents. The results shown in Figure 49 demonstrate that under these conditions these adducts had little or no effect on the accumulation of specific cleavage product.

Figure 50 shows the effects of adding gelatin to reactions in which the salt identity and concentration were varied from the standard reaction. In addition, all of these reactions were performed at 65°C, instead of 61°C. The reactions assayed in lanes 1-4 lacked added KCl, and included 0.02%, 0.05%, 0.1% or 0.2% gelatin, respectively. Lanes 5, 6, 7 and 8 contained the same titration of gelatin, respectively, and included 100 mM KCl. Lanes 9, 10, 11 and 12, also had the same titration of gelatin, and additionally included 150 mM LiCl in place of KCl. Lanes 13 and 14 show reactions that did not include gelatin, but which contained either 100 mM KCl or 150 mM LiCl, respectively. The results shown in Figure 50 demonstrated that in the absence of salt the gelatin had a moderately enhancing effect on the accumulation of specific cleavage product, but when either salt (KCl or LiCl) was added to reactions

performed under these conditions, increasing amounts of gelatin reduced the product accumulation.

j) Effect Of Adding Large Amounts Of Non-Target Nucleic Acid

In detecting specific nucleic acid sequences within samples, it is important to determine if the presence of additional genetic material (*i.e.*, non-target nucleic acids) will have a negative effect on the specificity of the assay. In this experiment, the effect of including large amounts of non-target nucleic acid, either DNA or RNA, on the specificity of the invasive cleavage reaction was examined. The data was examined for either an alteration in the expected site of cleavage, or for an increase in the nonspecific degradation of the probe oligonucleotide.

Figure 51 shows the effects of adding non-target nucleic acid (*e.g.*, genomic DNA or tRNA) to an invasive cleavage reaction performed at 65°C, with 150 mM LiCl in place of the KCl in the standard reaction. The reactions assayed in lanes 1 and 2 contained 235 and 470 ng of genomic DNA, respectively. The reactions analyzed in lanes 3, 4, 5 and 6 contained 100 ng, 200 ng, 500 ng and 1 μg of tRNA, respectively. Lane 7 represents a control reaction which contained no added nucleic acid beyond the amounts used in the standard reaction. The results shown in Figure 51 demonstrate that the inclusion of non-target nucleic acid in large amounts could visibly slow the accumulation of specific cleavage product (while not limiting the invention to any particular mechanism, it is thought that the additional nucleic acid competes for binding of the enzyme with the specific reaction components). In additional experiments it was found that the effect of adding large amounts of non-target nucleic acid can be compensated for by increasing the enzyme in the reaction. The data shown in Figure 51 also demonstrate that a key feature of the invasive cleavage reaction, the specificity of the detection, was not compromised by the presence of large amounts of non-target nucleic acid.